April 24, 2020

Personal Genome Diagnostics
Jennifer Dickey, Ph.D., RAC
VP, Regulatory and Quality
2809 Boston Street, Suite 503
Baltimore, Maryland 21224

Re: K192063
Trade/Device Name: PGDx™ elio tissue complete
Regulation Number: 21 CFR 866.6080
Regulation Name: Next generation sequencing based tumor profiling test
Regulatory Class: Class II
Product Code: PZM
Dated: July 31, 2019
Received: August 1, 2019

Dear Jennifer Dickey:

We have reviewed your Section 510(k) premarket notification of intent to market the device referenced above and have determined the device is substantially equivalent (for the indications for use stated in the enclosure) to legally marketed predicate devices marketed in interstate commerce prior to May 28, 1976, the enactment date of the Medical Device Amendments, or to devices that have been reclassified in accordance with the provisions of the Federal Food, Drug, and Cosmetic Act (Act) that do not require approval of a premarket approval application (PMA). You may, therefore, market the device, subject to the general controls provisions of the Act. Although this letter refers to your product as a device, please be aware that some cleared products may instead be combination products. The 510(k) Premarket Notification Database located at https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfpmn/pmn.cfm identifies combination product submissions. The general controls provisions of the Act include requirements for annual registration, listing of devices, good manufacturing practice, labeling, and prohibitions against misbranding and adulteration. Please note: CDRH does not evaluate information related to contract liability warranties. We remind you, however, that device labeling must be truthful and not misleading.

If your device is classified (see above) into either class II (Special Controls) or class III (PMA), it may be subject to additional controls. Existing major regulations affecting your device can be found in the Code of Federal Regulations, Title 21, Parts 800 to 898. In addition, FDA may publish further announcements concerning your device in the Federal Register.

Please be advised that FDA's issuance of a substantial equivalence determination does not mean that FDA has made a determination that your device complies with other requirements of the Act or any Federal statutes and regulations administered by other Federal agencies. You must comply with all the Act's requirements, including, but not limited to: registration and listing (21 CFR Part 807); labeling (21 CFR Part

801 and Part 809); medical device reporting (reporting of medical device-related adverse events) (21 CFR 803) for devices or postmarketing safety reporting (21 CFR 4, Subpart B) for combination products (see https://www.fda.gov/combination-products/guidance-regulatory-information/postmarketing-safety-reporting-combination-products); good manufacturing practice requirements as set forth in the quality systems (QS) regulation (21 CFR Part 820) for devices or current good manufacturing practices (21 CFR 4, Subpart A) for combination products; and, if applicable, the electronic product radiation control provisions (Sections 531-542 of the Act); 21 CFR 1000-1050.

Also, please note the regulation entitled, "Misbranding by reference to premarket notification" (21 CFR Part 807.97). For questions regarding the reporting of adverse events under the MDR regulation (21 CFR Part 803), please go to https://www.fda.gov/medical-devices/medical-device-safety/medical-device-reporting-mdr-how-report-medical-device-problems.

For comprehensive regulatory information about medical devices and radiation-emitting products, including information about labeling regulations, please see Device Advice (https://www.fda.gov/medical-devices/device-advice-comprehensive-regulatory-assistance) and CDRH Learn (https://www.fda.gov/training-and-continuing-education/cdrh-learn). Additionally, you may contact the Division of Industry and Consumer Education (DICE) to ask a question about a specific regulatory topic. See the DICE website (https://www.fda.gov/medical-devices/device-advice-comprehensive-regulatory-assistance/contact-us-division-industry-and-consumer-education-dice) for more information or contact DICE by email (DICE@fda.hhs.gov) or phone (1-800-638-2041 or 301-796-7100).

Sincerely,

Donna Roscoe, Ph.D.
Chief
Division of Molecular Genetics and Pathology
OHT7: Office of In Vitro Diagnostics and Radiological Health
Office of Product Evaluation and Quality
Center for Devices and Radiological Health

Enclosure

DEPARTMENT OF HEALTH AND HUMAN SERVICES
Food and Drug Administration

Indications for Use

Form Approved: OMB No. 0910-0120
Expiration Date: 06/30/2020
See PRA Statement below.

510(k) Number *(if known)*
K192063

Device Name
PGDx elio™ tissue complete

Indications for Use *(Describe)*
The PGDx elio™ tissue complete assay is a qualitative in vitro diagnostic device that uses targeted next generation sequencing of DNA isolated from formalin-fixed, paraffin-embedded tumor tissue from patients with solid malignant neoplasms to detect tumor gene alterations in a broad multi-gene panel.

PGDx elio tissue complete is intended to provide tumor mutation profiling information on somatic alterations (SNVs, small insertions and deletions, one amplification and four translocations), microsatellite instability (MSI) and tumor mutation burden (TMB) for use by qualified healthcare professionals in accordance with professional guidelines in oncology for previously diagnosed cancer patients, and is not conclusive or prescriptive for labeled use of any specific therapeutic product.

Type of Use *(Select one or both, as applicable)*

☒ Prescription Use (Part 21 CFR 801 Subpart D) ☐ Over-The-Counter Use (21 CFR 801 Subpart C)

CONTINUE ON A SEPARATE PAGE IF NEEDED.

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510(k) Summary

Submission Date: April 24, 2019

Submitter Information:

Submitted By: Personal Genome Diagnostics Inc.
2809 Boston Street, Suite 503
Baltimore, MD 21224

Contact Person: Jennifer S. Dickey PhD, RAC
Vice President, Regulatory & Quality
Personal Genome Diagnostics
Tel: (443) 602-8833
Email: jdickey@pgdx.com

A. Proprietary and Established Names

PGDx elio™ tissue complete

B. 510(k) number

K192063

C. Measurand

Somatic single nucleotide variants, insertions and deletions, select amplifications and translocations, microsatellite instability and tumor mutation burden in human genomic DNA obtained from formalin-fixed, paraffin-embedded tumor tissue.

D. Regulatory Information

1. Regulation section

21 CFR 866.6080

2. Classifications

Class II

3. Product Code

PZM

E. Indications for Use

1. Indications for Use

The PGDx elio™ tissue complete assay is a qualitative in vitro diagnostic device that uses targeted next generation sequencing of DNA isolated from formalin-fixed, paraffin-

embedded tumor tissue from patients with solid malignant neoplasms to detect tumor gene alterations in a broad multi-gene panel.

PGDx elio tissue complete is intended to provide tumor mutation profiling information on somatic alterations (SNVs, small insertions and deletions, one amplification and four translocations), microsatellite instability (MSI) and tumor mutation burden (TMB) for use by qualified healthcare professionals in accordance with professional guidelines in oncology for previously diagnosed cancer patients, and is not conclusive or prescriptive for labeled use of any specific therapeutic product.

2. **Special conditions for use statement(s):**

   For prescription use.

   For in vitro diagnostic use.

3. **Special Instrument Requirements**

   NextSeq® 550Dx (qualified by PGDx)

F. Substantial Equivalence Information:

1. **Predicate device name(s)**

   MSK-IMPACT

2. **Predicate 510(k) Number**

   DEN170058

3. **Comparison with predicate**

	PGDx elio tissue complete	MSK-IMPACT (Predicate Device)
K Number	K192063	DEN170058
SIMILARITIES		
Assay Intended Use / Indications for Use	The PGDx elio™ tissue complete assay is a qualitative in vitro diagnostic device that uses targeted next generation sequencing of DNA isolated from formalin-fixed, paraffin-embedded tumor tissue from patients with solid malignant neoplasms to detect tumor gene	The MSK-IMPACT assay is a qualitative in vitro diagnostic test that uses targeted next generation sequencing of formalin-fixed paraffin-embedded tumor tissue matched with normal specimens from patients with solid malignant neoplasms to detect tumor gene alterations in a broad multi gene panel. The test is intended to provide

	PGDx elio tissue complete	MSK-IMPACT (Predicate Device)
	alterations in a broad multi-gene panel. PGDx elio tissue complete is intended to provide tumor mutation profiling information on somatic alterations (SNVs, small insertions and deletions, one amplification and four translocations), microsatellite instability (MSI) and tumor mutation burden (TMB) for use by qualified healthcare professionals in accordance with professional guidelines in oncology for previously diagnosed cancer patients, and is not conclusive or prescriptive for labeled use of any specific therapeutic product.	information on somatic mutations (point mutations and small insertions and deletions) and microsatellite instability for use by qualified health care professionals in accordance with professional guidelines, and is not conclusive or prescriptive for labeled use of any specific therapeutic product. MSK-IMPACT is a single-site assay performed at Memorial Sloan Kettering Cancer Center.
Classification	II	Same
Product Code	PZM	Same
Regulation	21 CFR 866.6080	Same
Assay Method	Qualitative	Same
Sample Type	FFPE tumor tissue from cancer patients with solid malignant neoplasms	Same
Target Population	Previously diagnosed cancer patients with solid malignant neoplasms	Same
Mode of Measurement	PCR and Next Generation Sequencing (hybrid capture methodology)	Same
Calibrators	None	Same

	PGDx elio tissue complete	MSK-IMPACT (Predicate Device)
DNA Input	100 ng	100-250 ng
DIFFERENCES		
Test Environment	Kit	Single-site assay (performed at Memorial Sloan Kettering Cancer Center)
Controls	Positive control, negative control, normalized to database of common germline SNPs	Matched normal, positive control and negative control
Assay Target	• SNVs and indels in 505 genes • MSI • Amplification in ERBB2 • Translocations in ALK, RET, NTRK2, and NTRK3 • TMB	• SNVs and indels in 468 genes • MSI
Instrument	NextSeq 550Dx (qualified by PGDx)	HiSeq 2500 Sequencer (qualified by MSK)

G. Summary and Explanation

4. Product Description

PGDx elio tissue complete is an in vitro diagnostic assay that uses NGS to detect tumor gene alterations in genomic DNA isolated from formalin-fixed, paraffin-embedded (FFPE) tumor tissue from a variety of tumor types, using a targeted panel (505 genes). The assay takes less than 7 days from DNA to report and provides information on single nucleotide variants (SNVs) in a range of GC content and genomic contexts, insertion/ deletions (indels), 1 amplification as well as 4 translocations. It also identifies microsatellite instability based on select mononucleotide tracts and signatures of sequence mutations. The PGDx elio tissue complete assay utilizes a ~1.3 Mb region of interest (ROI) to calculate tumor mutation burden (TMB). **Figure 1.1** describes components of the assay. A complete list of components, equipment and materials can be found in Part 2 (User Guide) of the PGDx elio tissue complete Manual (MN-ETC-03). The panel gene list is provided in **Table 1.1**.

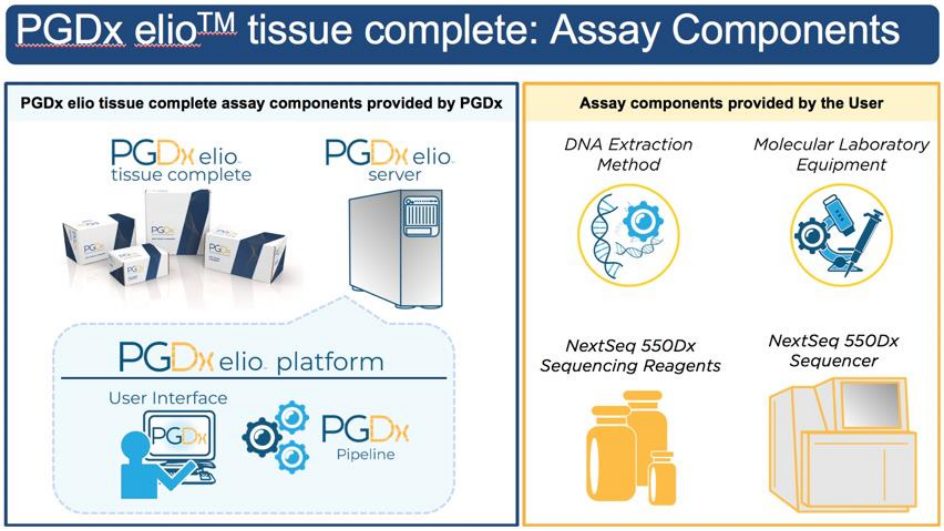

Figure 1.1 PGDx elio tissue complete assay components. PGDx provided components include reagent kits, software for data analysis, and a server.

Table 1.1

PGDx elio tissue complete Gene List‡							
ABL1	ABL2	ACVR1	ACVR1B	ADORA2A	AKT1	AKT2	AKT3
ALK*	ALOX12B	AMER1	APC	AR	ARAF	ARFRP1	ARID1A
ARID1B	ARID2	ARID5B	ASXL1	ASXL2	ATM	ATR	ATRX
AURKA	AURKB	AXIN1	AXIN2	AXL	B2M	BAP1	BARD1
BBC3	BCL2	BCL2L1	BCL2L11	BCL2L2	BCL6	BCOR	BCORL1
BCR	BIRC2	BLM	BMPR1A	BRAF	BRCA1	BRCA2	BRD4
BRIP1	BTG1	BTG2	BTK	BUB1B	C11ORF30	CALR	CARD11
CASP8	CBFB	CBL	CCND1	CCND2	CCND3	CCNE1	CD22
CD274	CD276	CD70	CD79A	CD79B	CDC73	CDH1	CDK12
CDK4	CDK6	CDK8	CDKN1A	CDKN1B	CDKN1C	CDKN2A	CDKN2B
CDKN2C	CEBPA	CHD2	CHD4	CHEK1	CHEK2	CIC	CREBBP
CRKL	CSF1	CSF1R	CSF2	CSF3	CSF3R	CTCF	CTLA4
CTNNA1	CTNNB1	CUL3	CUL4A	CXCR2	CXCR4	CYLD	CYP17A1
DAXX	DCUN1D1	DDB2	DDR1	DDR2	DICER1	DIS3	DNMT1

DNMT3A	DNMT3B	DOT1L	E2F3	EED	EGFL7	EGFR	EIF1AX
EP300	EPAS1	EPCAM	EPHA2	EPHA3	EPHA5	EPHA7	EPHB1
EPHB4	ERBB2#	ERBB3	ERBB4	ERCC1	ERCC2	ERCC3	ERCC4
ERCC5	ERCC6	ERCC8	ERG	ERRFI1	ESR1	ETV1	ETV4
ETV5	ETV6	EWSR1	EXT1	EXT2	EZH2	FAM175A	FAM46C
FANCA	FANCB	FANCC	FANCD2	FANCE	FANCF	FANCG	FANCI
FANCL	FANCM	FAS	FAT1	FBXW7	FGF10	FGF12	FGF14
FGF19	FGF23	FGF3	FGF4	FGF6	FGFR1	FGFR2	FGFR3
FGFR4	FH	FLCN	FLT1	FLT3	FLT4	FOXA1	FOXL2
FOXP1	FRS2	FUBP1	GABRA6	GATA1	GATA2	GATA3	GATA4
GATA6	GID4	GLI1	GNA11	GNA13	GNAQ	GNAS	GPC3
GPR124	GREM1	GRIN2A	GRM3	GSK3B	H3F3A	H3F3B	H3F3C
HDAC1	HDAC2	HDAC6	HGF	HIST1H1C	HIST1H2BD	HIST1H3B	HNF1A
HRAS	HSD3B1	HSP90AA1	HSP90AB1	ICOSLG	ID3	IDH1	IDH2
IFNGR1	IGF1	IGF1R	IGF2	IGF2R	IKBKE	IKZF1	IL10
IL7R	INHBA	INPP4A	INPP4B	INSR	IRF2	IRF4	IRS1
IRS2	JAK1	JAK2	JAK3	JUN	KAT6A	KDM5A	KDM5C
KDM6A	KDR	KEAP1	KEL	KIT	KLF4	KLHL6	KMT2A
KMT2C	KMT2D	KRAS	LATS1	LATS2	LMO1	LRP1B	LTK
LYN	LZTR1	MAF	MAGI2	MAML1	MAP2K1	MAP2K2	MAP2K4
MAP3K1	MAP3K13	MAPK1	MAX	MCL1	MDC1	MDM2	MDM4
MED12	MEF2B	MEN1	MERTK	MET	MITF	MKNK1	MLH1
MLH3	MPL	MRE11A	MSH2	MSH3	MSH6	MST1R	MTAP
MTOR	MUTYH	MYB	MYC	MYCL	MYCN	MYD88	MYOD1
NBN	NCOA3	NCOR1	NF1	NF2	NFE2L2	NFKBIA	NKX2-1
NKX3-1	NOTCH1	NOTCH2	NOTCH3	NOTCH4	NPM1	NRAS	NSD1
NT5C2	NTRK1	NTRK2*	NTRK3*	NUP93	NUTM1	PAK1	PAK3
PAK7	PALB2	PARK2	PARP1	PARP2	PARP3	PAX5	PAX8
PBRM1	PDCD1	PDCD1LG2	PDGFRA	PDGFRB	PDK1	PDPK1	PHOX2B
PIK3C2B	PIK3C2G	PIK3C3	PIK3CA	PIK3CB	PIK3CD	PIK3CG	PIK3R1
PIK3R2	PIK3R3	PIM1	PLCG2	PLK2	PMAIP1	PMS1	PMS2
PNRC1	POLD1	POLE	POLH	POT1	PPARG	PPP2R1A	PPP2R2A

PRDM1	PREX2	PRKAR1A	PRKCI	PRKDC	PRSS1	PRSS8	PTCH1
PTEN	PTK2	PTPN11	PTPRD	PTPRO	PTPRS	PTPRT	QKI
RAC1	RAD21	RAD50	RAD51	RAD51B	RAD51C	RAD51D	RAD52
RAD54B	RAD54L	RAF1	RANBP2	RARA	RASA1	RB1	RBM10
RECQL4	REL	RET*	RFWD2	RHOA	RICTOR	RIT1	RNF43
ROS1	RPA1	RPS6KA4	RPS6KB2	RPTOR	RUNX1	RUNX1T1	RYBP
SBDS	SDHA	SDHAF2	SDHB	SDHC	SDHD	SETD2	SF3B1
SGK1	SH2D1A	SHQ1	SLIT2	SLX4	SMAD2	SMAD3	SMAD4
SMARCA4	SMARCB1	SMARCD1	SMO	SNCAIP	SOCS1	SOX10	SOX17
SOX2	SOX9	SPEN	SPOP	SPTA1	SRC	STAG2	STAT3
STAT4	STK11	STK40	SUFU	SUZ12	SYK	TAF1	TBX3
TEK	TERC	TERT	TET1	TET2	TGFBR1	TGFBR2	TIPARP
TLR4	TLR7	TLR8	TLR9	TMEM127	TMPRSS2	TNFAIP3	TNFRSF14
TOP1	TOP2A	TP53	TP53BP1	TP63	TRAF7	TSC1	TSC2
TSHR	TYRO3	U2AF1	VEGFA	VHL	VTCN1	WAS	WEE1
WHSC1	WHSC1L1	WISP3	WRN	WT1	XIAP	XPA	XPC
XPO1	XRCC1	XRCC2	XRCC3	YAP1	YES1	ZBTB2	ZNF217
ZNF703							

‡ - All genes listed contribute to TMB score and SNV/indel reporting; * - Translocations reported for this gene; # - Amplifications reported for this gene

5. Sample Preparation

The PGDx elio tissue complete assay requires genomic DNA isolated from FFPE tissue specimens using commercially available DNA extraction methods. The assay is validated for use with DNA recovered from tissue with a minimum of 20% viable tumor nuclei. If less than 100% of the tissue section contains ≥20% tumor purity, the tissue should be macro-dissected to select as much viable tumor as possible and minimize the amount of adjacent non-tumor tissue. The recommended DNA input for the assay is 100 ng at a minimum concentration of 1 ng/µL; results can be obtained with inputs down to 50 ng.

6. Library Preparation

The PGDx elio tissue complete assay workflow begins with genomic DNA. Genomic DNA is quantified using a fluorometer. DNA molecules are mechanically sheared to a target size of 200 bp and subjected to a magnetic bead purification step to remove smaller fragments and perform an exchange of buffer.

Fragmented DNA is end-repaired, phosphorylated, and adenylated. Indexed adapters are then ligated to the A-tailed DNA molecules. Unincorporated adapters and reagents are removed by magnetic bead purification. Adapter-ligated DNA is enriched by PCR amplification. Primer dimers and residual reagents are removed by magnetic bead purification. Library quality is assessed using a DNA fragment analyzer prior to hybrid capture.

7. Hybrid Capture NGS

The adapter-ligated library is hybridized with biotinylated RNA library baits, and targeted regions are captured using magnetic streptavidin coated beads. Captured libraries are purified to remove baits and incompletely hybridized DNA fragments. Captured libraries are enriched by PCR amplification. Primer dimers and residual reagents are removed by magnetic bead purification. Final library quality is assessed using a DNA fragment analyzer prior to sequencing.

8. Sequencing

Sample libraries are quantified and normalized into a sequencing pool of up to 15 samples and the external control. Pooled sample libraries are fluorometrically quantified, loaded on a sequencing flow cell and sequenced using a NextSeq® 550Dx instrument which has been pre-qualified by PGDx.

9. Data Analysis

Sequence data is processed using the PGDx elio platform software. The software contains a user interface that tracks sample status from sequencing through analysis and reporting. Users configure sequencing runs, and an automated pipeline of software for bioinformatic analysis identifies and reports genomic alterations. After processing, the software generates FASTQ files containing sequences and quality scores for each sample. The FASTQ files are then aligned to a reference genome to generate BAM files, which are processed for variant calling of different alteration types (SNVs, indels, amplifications, translocations, and MSI). SNVs and indels are then used to determine TMB scores reported as mutations per megabase.

10. Controls

i. Negative Control: A no template control (NTC) can be processed to serve as a negative control to validate the acceptability of all the test samples processed through library preparation and capture steps by testing for sample or reagent contamination. The NTC is not included on the sequencing run.

ii. Positive Control: An external control that is provided in the PGDx elio tissue complete assay reagent kit consists of cell line derived-DNA with multiple verified sequence mutations. The external control is processed from library preparation through sequencing to serve as an end to end control to demonstrate assay

performance. The external control is checked for quality during library preparation and after sequencing. Failure of the external control to meet the pre-defined quality metrics will result in all test samples on the run being reported as 'No result.'

11. Results Reporting

PGDx elio tissue complete reports SNVs and indels in protein coding regions across all genes in the panel. In addition, amplifications are reported for ERBB2 as well as translocations for ALK, RET, NTRK2, and NTRK3. The assay also reports on 2 genomic signatures, MSI and TMB.

The variants listed in the section Variants with Evidence of Clinical Significance are determined based on the selected tumor type. Only variants clinically associated with the tested tumor type will appear in the Variants with Evidence of Clinical Significance section. Any remaining detected variants will appear as the Variants with Potential Clinical Significance. A qualified healthcare professional can select the appropriate tumor type, and depending on the tumor type selected, variants will be reported as Variants with Evidence of Clinical Significance by PGDx elio tissue complete. Any variants clinically associated with tumor types other than the one selected will be reported in the section labeled 'Variants with Potential Clinical Significance**.** A list of all genes is provided in **Appendix A.**

SNVs and indels results are also presemted in terms of somatic hotspot or non-hotspot. PGDx defines hotspots as > 25 exact hits in COSMIC version 72. A lower minimum MAF is used when reporting these hotspot mutations.

Table 1: Summary of PGDx elio tissue complete Quality Control Metrics Post-sequencing

Quality Metric	Level of Qualification	Passing Criteria
Cluster Density	Batch-level	Sequencer Cluster Density ≥ 130
Q30 Reads	Batch-level	%Q30 (Read1 and Read4) ≥ 80% %Q30 (Read2 and Read3) ≥ 85%
External Control	Batch-level	All expected sequence mutations are detected and passes all other quality criteria
Percent Regions Covered	Sample-level	≥ 90% exons with > 100x Median Distinct Coverage
Percent Reads Identified	Sample-level	Percent Reads Identified 15%-35%
Contamination QC	Sample-level	Estimated contamination levels < 2%

Quality Metric	Level of Qualification	Passing Criteria
Select SNVs and Indels with Evidence of Clinical Significance	Analyte-level	Mutant reads ≥ 4 MAF > 0.4%
Hotspot SNVs and Indels	Analyte-level	Mutant reads ≥ 4 MAF > 2%
Non-hotspot SNVs	Analyte-level	Mutant reads ≥ 6 MAF with lower bound 95% CI ≥ 5%
Non-hotspot Indels	Analyte-level	Mutant reads ≥ 6 MAF > 5%
Homopolymer Indels	Analyte-level	Homopolymer regions < 5 bp or Homopolymer regions ≥ 5 bp with MAF ≥ 12%
ERBB2 Amplifications	Analyte-level	Fold change ≥ 2.5 in ≥ 25% regions covered
Translocations (ALK, NTRK2, NTRK3 and RET)	Analyte-level	Fusion reads ≥ 3

12. Analytical Studies

13. Specificity

i. Limit of Blank (LoB)

Non-cancerous FFPE tissues were assessed for analytical specificity to confirm the reporting thresholds and quality metrics minimize false positives. Two reference standards from National Institute of Standards and Technology (NIST), NA24531 and NA24385 were evaluated by PGDx elio tissue complete for variants reported at the 100ng DNA input. Specificity was observed at 100% with no unverified mutations reported across 5 replicates for each standard. Unique test cases from normal FFPE samples were processed with the recommended 100 ng DNA input across 2 different lots of the PGDx elio tissue complete assay kit. For Variants with Evidence of Clinical Significance, the rate of false positives is < 0.1% while the false positive rate for hotspot SNVs is < 3.2% (n=2/63). For MSI-H, the false positive rate is < 1.6%.

ii. Cross Reactivity

An in silico cross-reactivity analysis was performed to evaluate specificity of capture baits based on sequence identity of target baits to the human reference genome. Bait sequences corresponding to gene regions of clinical significance demonstrated specificity to target regions based on mapping quality or unique sequence identity to the human reference genome. Any bait that had some sequence identity to regions other than the target region of interest was further investigated and were shown to be specific. This study demonstrated that the oligonucleotide baits in the PGDx elio tissue complete assay are specific to the target regions of DNA intended to be reported.

14. Sensitivity

i. Limit of Detection (LoD) – SNVs and Indels

The LoD was assessed by variant type and is defined as the lowest MAF at which ≥95% of replicates are detected. The LoD was evaluated 2 ways, through a dilution series using cell lines to determine the LoD and by confirmation with clinical specimens. Ten unique clinical cases were selected for SNVs, insertions and deletions and diluted with normal DNA derived from FFPE tissues. Each unique clinical case was tested with 10 replicates across 2 kit lots (n=20 per specimen) for a total of 200 observations (Error! Reference source not found.). Data was aggregated across 2 reagent kit lots when possible, otherwise the lot with the higher MAF LoD is displayed.

Additional evaluations of analytical sensitivity performance used dilution series of FFPE clinical specimens. The positive call rates were assessed for a total of 11 SNVs, 3 insertions, and 5 deletions from 5 clinical FFPE specimens with 5 replicates per dilution level. A range of 5.9-12.6% MAF was observed using the lowest average MAF where the positive call rates was ≥ 95%.

Cell lines were used to establish the LoD MAF range for 451 SNVs and 31 indels across the panel. A total of 150 observations were generated (3 samples with 10 replicates at 5 dilution levels). The established ranges were then confirmed with a ≥ 95% call rate with FFPE clinical cases on a per variant level (Error! Reference source not found.) and for the entire panel (**Table 1.2**). A summary of the LoD by variant type across all replicates is shown in the table below.

Table 1.2 Analytical Sensitivity (LoD MAF) for Representative SNVs and Indels

Variant	MAF Range	Cell Line Variants Evaluated to Establish MAF Range	Number of Variants in Clinical Cases in the Established Range
Hotspot SNVs	3.1% to 5.4%	8	2
Non-hotspot SNVs	6.3% to 17.8%	443	176
Indels at homopolymer context[1]	13.7% to 17.5%	10	9

Indels at non-homopolymer context	6.1% to 10.9%	19	4

[1] Greater than or equal to 5 bp repeat

ii. LoD – ERBB2, ALK, RET, NTRK2, NTRK3 and MSI

Analytical sensitivity of ERBB2, ALK, RET, NTRK3, and MSI was confirmed by testing 7 clinical FFPE cases diluted with normal FFPE DNA to achieve targeted detection levels. Each unique case was confirmed at ≥ 95% call rate at 1 tumor purity level, with 10 replicates per kit lot, across 2 unique lots for translocations and amplifications. For MSI-H, 3 cases were confirmed at 1 tumor purity level with 10 replicates each. Results summarized in **Table 1.3** indicate that the assay is sensitive in detecting specific translocations, amplifications and MSI-H.

Table 1.3 Analytical Sensitivity (LoD Tumor Purity) – Translocations, Amplifications and MSI

Variant	LoD Tumor Purity
MSI-H	18.1%
ERBB2 amplifications	4.4%
ALK translocations[1]	5.6%
NTRK2 translocations	30%[2]
NTRK3 translocations	11.5%
RET translocations	12.8%

[1] The enrolled ALK case was evaluated with only 17 total replicates due to insufficient DNA quantity.

[2] In silico down sampling suggests LoD 3%.

iii. TMB and Tumor Purity

The minimum tumor purity requirement for input into PGDx elio tissue complete is 20%. The minimum tumor purity required for robust reporting of TMB scores by PGDx elio tissue complete was established using 8 clinical FFPE cases. Samples 1-5 were serially diluted across 3 levels with 5 replicates per level and 1 level with 3 replicates (18 total), sample 6 was serially diluted across 5 levels at 10 replicates per level (50 total), and samples 7-8 were serially diluted across 5 levels with 5 replicates per level (25 total, 10 of which had a tumor purity ≥15%). The total number of replicates per sample and the %CV of all replicates with ≥15% tumor purity are shown below in **Table 1.4**. Together these data show PGDx elio tissue complete TMB performance across tumor purities at or above 15%. **Figure 1.2** demonstrates the consistency in TMB as a function of tumor purity for each of the specimens assessed across a range of TMB Muts/Mb scores.

Table 1.4 TMB Precision for Samples ≥ 15% Tumor Purity

Sample	Reference Undiluted TMB Score	CV of Replicates ≥ 15% Tumor Purity	Number of Replicates ≥ 15% Tumor Purity
1	33.4	12.5%	18
2	24.8	10.5%	18
3	50.8	6.3%	18
4	64.7	15.9%	18
5	31.5	8.0%	18
6	455.4	3.3%	50
7	10.0	14.8%	10
8	14.6	6.0%	10

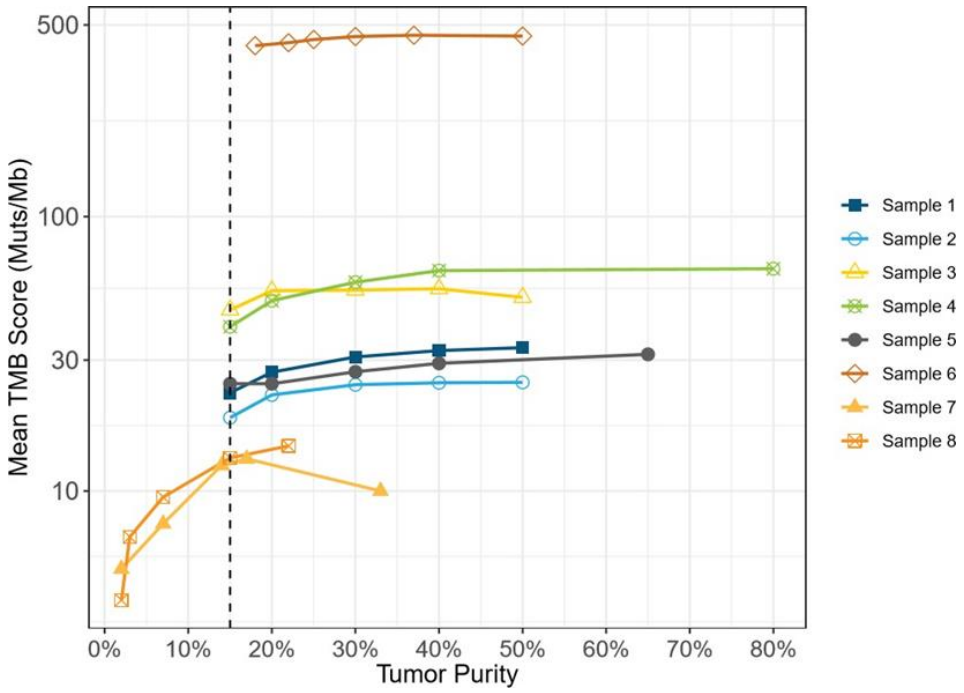

Figure 1.2 Linearity of TMB score with tumor purity in PGDx elio tissue complete. The tumor purity is shown on the x-axis and the mean TMB score of the replicates at a specific tumor purity is shown on the y-axis.

iv. DNA Extraction

PGDx elio tissue complete is compatible with genomic DNA extracted from FFPE samples using any appropriate commercially available FFPE extraction method. Samples (3 FFPE specimens and 1 cell line) were extracted in duplicate by 2 operators using 3 different methods, equaling 48 total samples and processed in duplicate for a total of 96 observations to assess concordance. Data for all variant types, including BRAF V600

SNV, ALK and NTRK3 translocations and ERBB2 amplification, were aggregated and Positive Percent Agreement (PPA) and Negative Percent Agreement (NPA) are presented in **Table 1.5**. Method 2 (bead-based) and Method 3 (automated) were compared to the reference Method 1 (column-based). The overall pass rate for FFPE samples was 93.1% (67/72). The %CV for TMB in assessed cases was < 12.5%. All DNA extraction methods yielded concordant results in variant calls with PGDx elio tissue complete.

Table 1.5 DNA Extraction Methods Compared to Reference

DNA Extraction Method	Concordance in Variant Calls with Method 1 (n/N) (2-sided 95% CI)
Method 2	PPA - 97.8% (673/688) (96.4%, 98.7%)
	NPA - 99.9% (71535481/71535520) (99.9%, 100%)
Method 3	PPA - 97.5% (624/640) (96.0%, 98.5%)
	NPA - 99.9% (51416128/51416155) (99.9%, 100%)

v. DNA Input

The recommended DNA input for PGDx elio tissue complete is 100 ng. To evaluate assay performance across a range of DNA inputs, 4 unique FFPE samples with known variants were prepared in triplicate at 10, 25, 50, 100, and 200 ng DNA input levels. The variant calls for these samples were compared to the respective reference DNA input of 100 ng for each case to assess concordance. **Table 1.6** describes PPA and NPA for each input level where aggregated variants were analyzed, including SNVs, indels, amplifications, translocations, and MSI. For TMB, the mean absolute percent error rate of 10, 25, 50 and 200 ng DNA input compared to 100 ng were 11.8%, 3.3%, 4.4% and 1.8%, respectively. These data indicate the assay is robust around the recommended 100 ng DNA input.

Table 1.6 DNA Input Compared to 100 ng Reference

DNA Input	Variant Call Concordance (n/N) (2-sided 95% CI)
10 ng	PPA - 92.2% (177/192) (87.5%, 95.2%)
	NPA - 99.9% (26825815/26825826) (99.9%, 100%)
25 ng	PPA - 94.8% (182/192) (90.7%, 97.1%)
	NPA - 99.9% (26825815/26825826) (99.9%, 100%)
50 ng	PPA - 96.9% (186/192) (93.4%, 98.6%)
	NPA - 99.9% (26825822/26825826) (99.9%, 100%)
200 ng	PPA - 97.4% (187/192) (94.0%, 98.9%)
	NPA - 99.9% (26825818/26825826) (99.9%, 100%)

vi. Sample Carryover and Cross-contamination

Cross-contamination (contamination from one sample to another within the same batch) and sample carryover (contamination from a previous sequencing run when using the same instrument) were assessed by evaluating false positive and false negative variant calls in 29 FFPE samples. Seven of the 29 cases had known positive variants, the remaining samples were known negative samples. All FFPE samples were assessed across 2 batches to test for contamination within and between runs. In batch 1, a checkerboard pattern within a 96-well plate was created by alternating the samples with representative positive variants and known negative samples. Batch 2 contained known negative samples and was pooled and sequenced directly after completion of batch 1 sequencing, following standard instrument cleaning procedures. No positive variant results were observed in known negative samples tested. Therefore, the PGDx elio tissue complete assay workflow presents minimal risk for contamination.

15. Interference (Endogenous and Exogenous)

i. Interfering Substances (Exogenous)

The impact of exogenous interfering substances on the performance of the PGDx elio tissue complete assay was assessed by processing DNA from FPPE samples tested in the presence of each interfering substance at varying amounts (**Table 1.7**). The samples were evaluated for concordance of variant calls when compared to samples processed without the interfering substances. Replicates for 5 test cases were analyzed for 8 experimental and 2 baseline conditions. Analysis of all variant types tested (SNVs, indels, translocations, amplifications and MSI) showed high PPA (> 97.2%) and NPA (>99.9%) for all variants. The TMB mean absolute percent error (MAPE) ranged from 0% to 6.0% across conditions. The results show minimal risk to assay performance from interfering exogenous substances.

Table 1.7 Exogenous Interfering Substances Tested

Substance	Amount in Excess of Standard Conditions
Proteinase K	2X and 3X
Indexed adapters	15% and 30%
Melanin	0.2 µg/mL and 1.6 µg/mL
Ethanol	2.5% and 5%

ii. Endogenous Interference

The impact of necrosis and FFPE block age on the performance of PGDx elio tissue complete was evaluated by assessing the first pass and overall pass rates of samples processed in the accuracy study (see Accuracy section below). Of 521 samples enrolled for accuracy, 448 were evaluated for necrosis over a range of 0-75%, and 378 were evaluated for age of block over a range of 0-253 months. The data indicated there is no

correlation between necrosis and pass rate. A logistic regression analysis was performed to establish the probability of sample pass/fail according to FFPE block age as shown in **Figure 1.3**. There is a correlation between overall pass rate and the age of block; as the age of block increases, the overall pass rate decreases. The probability of samples passing from blocks aged roughly 175 months (or 14.5 years) is approximately 75%. The results show minimal risk to assay performance from interfering endogenous factors.

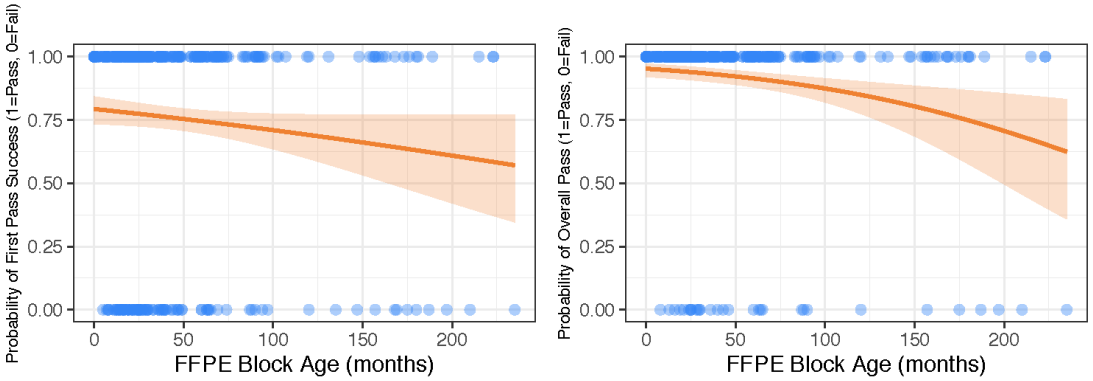

Figure 1.3 Logistic Regression Graphs of First Pass and Overall Acceptability vs FFPE Block Age. The orange line represents a regression line, and orange shading represents the 95% confidence interval. The blue dots represent individual samples assessed.

16. Assay Acceptance Rates

Multiple factors can influence overall robustness and performance of complex molecular tests, including pre-analytical factors and overall sample quality. If key in-process or automated data quality metrics are not met, PGDx elio tissue complete supports repeating samples through the workflow. Performance throughout verification and validation of the device was tracked and a summary of the rates for first pass (no repeat) and overall pass (allowing for a single repeat) are presented below.

i. Overall Clinical FFPE Sample Acceptance Rate

Data were aggregated for unique clinical cases from >40 tumor types assessed during verification and validation of PGDx elio tissue complete. Resulting pass rates for clinical samples are presented in **Table 1.8**. The data indicate that results are obtained on a high percentage of FFPE samples after processing through the PGDx elio tissue complete assay.

Table 1.8 PGDx elio tissue complete Acceptability Rates

First Pass Rate (n/N) (2-sided 95% CI)	Overall Pass Rate (n/N) (2-sided 95% CI)
81.8% (2352/2874) (80.4%, 83.2%)	92.9% (2671/2874) (91.9%, 93.8%)

ii. Pan-Tumor Type/Tissue Comparability

Invalid rates for the different tumor types assessed in the analytical accuracy study are provided in **Table 1.9** below.

Table 1.9 Specimen Invalid Rates for >18 FFPE Tumor Types

Tumor Type	Passing Samples	Total Samples	Invalid Rate (%)
Bladder	6	7	14.3
Brain	10	10	0
Breast	60	72	16.7
Colorectal	91	97	6.2
Endometrial	27	27	0
Gastric	25	31	19.4
Glioma	4	4	0
Head and Neck	5	6	16.7
Lung – NOS[1]	64	68	5.9
Melanoma	34	36	5.6
NOS[1]	8	8	0
NSCLC[1]	85	92	7.6
Other[2]	21	22	4.5
Ovarian	8	9	11.1
Pediatric Glioma	9	9	0
Prostate	7	8	12.5
Skin	4	4	0
Triple Negative Breast	11	11	0
Total	479	521	8.1

[1]NOS: not otherwise specified; NSCLC: non-small cell lung cancer.

[2]Other ($n \leq 3$ cases per tumor type): cervical, cholangiocarcinoma, gallbladder, pancreatic, rhabdomyosarcoma, trachea, esophageal, fallopian tube, liver, mediastinum, peritoneal, renal, and thyroid.

17. Accuracy – Concordance to Orthogonal Methods

In order to demonstrate the accuracy of PGDx elio tissue complete as a tumor profiling device, a study was performed with 582 samples that had both PGDx elio tissue complete data and orthogonal data. Due to the rarity of specific genetic variants in solid tumor FFPE samples, most samples selected for this study were pre-screened, resulting in enrichment of certain variants relative to real-world clinical prevalence. Data were

aggregated at the variant level for SNVs, insertions, and deletions, gene level for amplifications and translocations, and case level for MSI and TMB.

The results summarized in **Table 1.10** indicate that the assay accurately detects SNVs and indels.

Table 1.10 Accuracy – SNVs and Indels

Variant	Orthogonal Method(s)	Performance (n/N) (2-sided 95% CI)
SNVs with Evidence of Clinical Significance	2 NGS targeted panels	PPA – 97.2% (35/36) (85.8%, 99.5%)
		NPA – 99.9% (3994/3996) (99.8%, 99.9%)
Hotspot SNVs	2 NGS targeted panels and PCR	PPA – 97.1% (132/136) (92.7%, 98.9%)
		NPA – 99.9% (35845/35850) (99.9%, 99.9%)
Non-hotspot SNVs	2 NGS targeted panels	PPA – 85.1% (516/606) (82.1%, 87.8%)
		NPA – 99.9% (178513452/178513618) (99.9%, 99.9%)
SNVs with Potential Clinical Significance	2 NGS targeted panels	PPA – 86.4% (591/684) (83.6%, 88.8%)
		NPA – 99.9% (178513372/178513540) (99.9%, 99.9%)
Hotspot deletions	2 NGS targeted panels and PCR	PPA – 100% (20/20) (84.5%, 100%)
		NPA – 99.9% (2064/2067) (99.6%, 99.9%)
Hotspot insertions	2 NGS targeted panels	PPA – 100% (1/1) (20.7%, 100%)
		NPA – 100% (2015/2015) (99.8%, 100%)
Non-hotspot indels	NGS targeted panel	PPA – 81.4% (79/97) (72.6%, 87.9%)
		NPA – 99.9% (67104842/67104857) (99.9%, 99.9%)
Non-hotspot insertions	NGS targeted panel	PPA – 80.8% (21/26) (62.1%, 91.5%)
		NPA – 99.9% (67104926/67104928)
Non-hotspot deletions	NGS targeted panel	PPA – 81.7% (58/71) (71.2%, 89.0%)
		NPA – 99.9% (67104870/67104883)
Insertions with Potential Clinical Significance	NGS targeted panel	PPA – 80.8% (21/26) (62.1%, 91.5%)
		NPA – 99.9% (67497962/67497964) (99.9%, 99.9%)

Deletions with Potential Clinical Significance	NGS targeted panel	PPA – 82.7% (62/75) (72.6%, 89.6%)
		NPA – 99.9% (67497902/67497915) (99.9%, 99.9%)

a) Accuracy for ERBB2 Amplifications

Concordance for ERBB2 Amplifications was assessed by comparing PGDx elio tissue complete to ERBB2 FISH (**Table 1.11** and **Table 1.12**). The PPA was 75.0% (95% CI: 62.3%, 84.5%) for all cases and 87.0% (95% CI: 74.3%, 93.9%) when excluding borderline FISH cases (defined as HER2/CEP17 ratio between 1.5 and 2.5). The NPA was 96.7% (95% CI: 90.8%, 98.9%) for all cases and 95.9% (95% CI: 88.7%, 98.6%) when excluding borderline FISH cases.

Table 1.11 Summary of Concordance between PGDx elio tissue complete and ERBB2 FISH Including Borderline Cases

	ERBB2 FISH		
PGDx elio tissue complete	ERBB2 Positive	ERBB2 Negative	Total
ERBB2 Positive	42	3	45
ERBB2 Negative	14	88	102
Total	56	91	147

Table 1.12 Summary of Concordance between PGDx elio tissue complete and ERBB2 FISH Excluding Borderline Cases

	ERBB2 FISH		
PGDx elio tissue complete	ERBB2 Positive	ERBB2 Negative	Total
ERBB2 Positive	40	3	43
ERBB2 Negative	6	71	77
Total	46	74	120

b) Accuracy for ALK Translocations

Concordance for ALK translocations was assessed by comparing PGDx elio tissue complete to ALK FISH (**Table 1.13**). The PPA was 92.9% (95% CI: 68.5%, 98.7%), and NPA was 98.2% (95% CI: 90.7%, 99.7%).

Table 1.13 Summary of Concordance between PGDx elio tissue complete and ALK

FISH

	ALK FISH		
PGDx elio tissue complete	ALK Positive	ALK Negative	Total
ALK Positive	13	1	14
ALK Negative	1	56	57
Total	14	57	71

ALK translocations were additionally assessed in silico due to limited availability of clinical cases close to the ALK FISH equivocal zone (10%-50% rearrangement positive nuclei). A total of 410 observations were generated for ALK by down-sampling 10 clinical samples from analytical accuracy to 4 tumor purity dilution levels with 10 replicates per level, to mimic samples in the FISH equivocal zone. For example, if the undiluted sample had a FISH score of 50% from analytical accuracy, the sample was diluted with wild type reads by a factor of 0.8 to get to a 40% positive nuclei FISH score. These data demonstrate an 88% positive call rate at 20% positive nuclei by FISH (**Table 1.14**).

Table 1.14 In silico Analysis of ALK Translocation Borderline Performance

FISH (%Positive Nuclei)	FISH Call	PGDx elio tissue complete Positive Call Rate (%) (n/N) (95% CI)
50 - 88	+	100% (10/10) (72%, 100%)
40	+	98% (98/100) (93%, 99%)
30	+	95% (95/100) (89%, 98%)
20	+	88% (88/100) (80%, 93%)
10	-	80% (80/100) (71%, 87%)

c) Accuracy for RET Translocations

Concordance for RET translocations was assessed by comparing PGDx elio tissue complete to RET FISH (**Table 1.15**). The PPA was 55.6% (95% CI: 26.7%, 81.1%), and NPA was 100% (95% CI: 82.4%, 100%).

Table 1.15 Summary of Concordance between PGDx elio tissue complete and RET FISH

	RET FISH		
PGDx elio	RET Positive	RET Negative	Total
RET Positive	5	0	5
RET Negative	4[1]	18	22

Total	9	18	27

[1]No read data supporting RET translocations was found in the raw data for the discrepant cases. In addition to the clinical samples processed to assess RET translocations, 3 RET translocation-positive cell lines were also tested with PGDx elio tissue complete. All 3 cell lines were positive for a fusion either by a validated assay performed by the cell line provider, or via literature. PGDx elio tissue complete detected all 3 fusions in these cell lines.

ii. Accuracy – TMB

The PGDx elio tissue complete assay reports a TMB score comprised of sequence mutations detected across the entire coding region of interest per sample. The ability of PGDx elio tissue complete to accurately identify TMB in multiple solid tissue FFPE tumor types was assessed by comparing to matched tumor-normal whole exome sequencing results. Across 8 tumor types (non-small cell lung carcinoma (NSCLC), melanoma, renal, bladder, endometrial, triple negative breast, head and neck, lung-NOS (not otherwise specified)), 118 cases were enrolled covering a dynamic range of 1.5-118.5 Muts/Mb. Of those, 31 fell below the established LoB (≤ 7.2 Muts/Mb). The Spearman correlation coefficient was used to determine the relationship between the 2 assays. Assessment of all 118 cases resulted in a Spearman correlation coefficient of 0.903. The results in **Figure 1.4** show strong concordance between PGDx elio tissue complete TMB scores and tumor-normal whole exome sequencing.

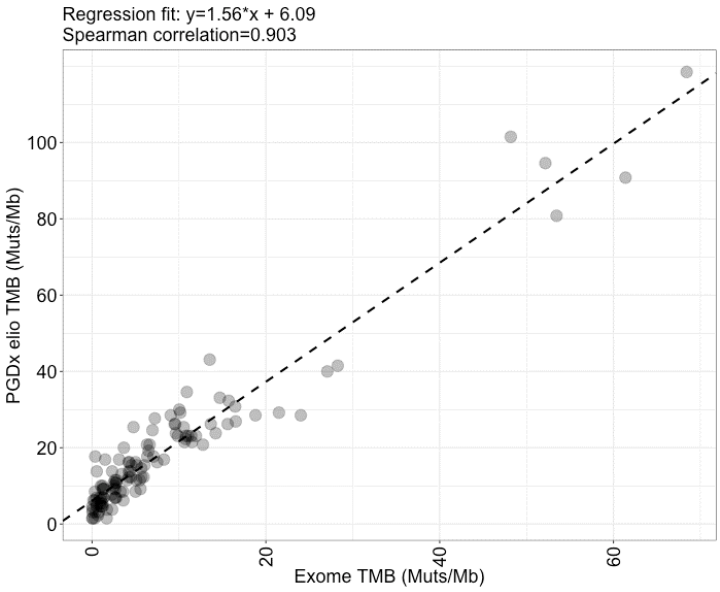

Figure 1.4 PGDx elio tissue complete TMB score vs. Matched Tumor-Normal Exome Sequencing.

iii. Accuracy – MSI

Table 1.16 contains MSI performance for all 283 samples including PGDx elio tissue complete and PCR failures and indeterminates. This cohort is further divided into performance for colorectal (CRC) and endometrial cases (**Table 1.17**) and non-CRC and non-endometrial cases (**Table 1.18**). MSI accuracy was assessed in 18 tumor types: ampulla (1), bladder (7), breast (21), colorectal (66), endometrial (18), esophagus (1), fallopian tube (1), gall bladder (1), gastric (40), lung (39), kidney (3), omentum (1), ovarian (2), prostate (4), sarcoma (3), skin (8), thyroid (2), and cancer of unknown primary (5).

Table 1.16 MSI Performance for All Cases

		MSI PCR				Total
		MSI	MSS	Failed	Indeterminate	
PGDx elio tissue complete	MSI	79	1	0	1[1]	81
	MSS	1	142	0	5[2]	148
	Failed	4	40	10	0	54
Total		84	183	10	6	283

Excluding failed/ indeterminate specimens with 95% CI	PPA	98.8% (79/80) (93.3%, 99.8%)
	NPA	99.3% (142/143) (96.1%, 99.9%)
	PPV	98.8% (79/80) (93.3%, 99.8%)
	NPV	99.3% (142/143) (96.1%, 99.9%)
Accounting for failed/ indeterminate specimens with 95% CI	PPA	94.0% (79/84) (86.8%, 97.4%)
	NPA	77.6% (142/183) (71.0%, 83.0%)
	PPV	97.5% (79/81) (91.4%, 99.3%)
	NPV	95.9% (142/148) (91.4%, 98.1%)

[1]This case was MSI-H by PGDx elio, and Promega PCR gave an “Indeterminate” result.

[2]These 5 cases did not have matching normal DNA to test via Promega PCR.

Table 1.17 MSI Performance for CRC and Endometrial Cases

		MSI PCR				Total
		MSI	MSS	Failed	Indeterminate	
PGDx elio tissue complete	MSI	51	0	0	0	51
	MSS	0	33	0	0	33
	Failed	0	0	0	0	0
Total		51	33	0	0	84

Excluding failed/ indeterminate	PPA	100% (51/51) (93.0%, 100%)
	NPA	100% (33/33) (89.6%, 100%)

specimens with 95% CI	PPV	100% (51/51) (93.0%, 100%)
	NPV	100% (33/33) (89.6%, 100%)
Accounting for failed/ indeterminate specimens with 95% CI	PPA	100% (51/51) (93.0%, 100%)
	NPA	100% (33/33) (89.6%, 100%
	PPV	100% (51/51) (93.0%, 100%)
	NPV	100% (33/33) (89.6%, 100%

Table 1.18 MSI Performance for Non-CRC and Non-Endometrial Cases

		MSI PCR				Total
		MSI	MSS	Failed	Indeterminate	
PGDx elio tissue complete	MSI	28	1	0	1[1]	30
	MSS	1	109	0	5[2]	115
	Failed	4	40	10	0	54
Total		33	150	10	6	199

Excluding failed/ indeterminate specimens with 95% CI	PPA	96.6% (28/29) (82.8%, 99.4%)
	NPA	99.1% (109/110) (95.0%, 99.8%)
	PPV	96.6% (28/29) (82.8%, 99.4%)
	NPV	99.1% (109/110) (95.0%, 99.8%)
Accounting for failed/ indeterminate specimens with 95% CI	PPA	84.8% (28/33) (69.1%, 93.4%)
	NPA	72.7% (109/150) (65.0%, 79.2%)
	PPV	93.3% (28/30) (78.7%, 98.2%)
	NPV	94.8% (109/115) (89.1%, 97.6%)

[1]This case was MSI-H by PGDx elio, and Promega PCR gave an "Indeterminate" result.

[2]These 5 cases did not have matching normal DNA to test via Promega PCR.

iv. Method Comparison Study for Wild Type Calls

A study was conducted to assess accuracy for 75 hotspot loci within 20 genes. A total of 112 specimens were tested, and the accuracy of PGDx elio tissue complete at all 75 positions was compared to 2 orthogonal methods (42 samples using 1 method, and 70 using a second method). Within the 112 specimens, there were 112 mutations across samples and 8,283 wild type calls. Overall variant-level concordance (PPA and NPA) was 96.4% and 99.9% respectively with two-sided 95% confidence intervals of (91.1%, 99.0%) for mutations (PPA), and (99.9%, 99.9%) for wild type locations (NPA).

18. Reproducibility

i. Interlaboratory Reproducibility

Interlaboratory reproducibility of the PGDx elio tissue complete assay was assessed across 3 different sites, using DNA extracted from 13 FFPE tissue specimens and 1 cell line. Together these 14 samples represented a range of SNVs and indels, ERBB2 amplifications, ALK, RET, and NTRK3 translocations, MSI, and TMB. Each of the 14 samples was tested in duplicate by 2 different operators on 12 sequencing runs across 3 non-consecutive days at each of the 3 independent laboratory sites using a single kit lot. The first pass rate was 90.3% (455/504) and the overall pass rate of the study was 98.2% (495/504) allowing a maximum of 1 round of repeat testing. Reproducibility was assessed 3 ways; (1) agreement for each positive variant detected across all replicates is reported (Positive call rate), and (2) Average Positive Agreement (APA) and Average Negative Agreement (ANA) and (3) modal analysis was used for per specimen reproducibility.

The positive call rate across all variants was 86.2%, while for SNVs, insertions, and deletions it was 88.8%, 82.8%, and 80.5%, respectively. **Table 1.19** shows the positive call rate stratified by variant type and allele fraction.

Table 1.19 Interlaboratory Reproducibility Call Rates

Mutation Type	MAF Threshold	Positive Call Rate Among All Observed Mutations	Total Unique Variants
All	MAF≥0	86.2% (14493/16813)	474
All	MAF≥5	88.0% (14483/16458)	464
All	MAF≥8	91.9% (13921/15146)	427
All	MAF≥10	93.1% (13404/14400)	406
All	MAF≥15	96.4% (12387/12846)	362
All SNVs	MAF≥0	88.4% (10549/11937)	337
All SNVs	MAF≥5	91.0% (10539/11582)	327
All SNVs	MAF≥8	95.7% (10070/10519)	297
All SNVs	MAF≥10	97.7% (9618/9845)	278
All SNVs	MAF≥15	97.8% (8773/8966)	253
All Insertions	MAF≥0	82.8% (649/784)	22
All Insertions	MAF≥5	82.8% (649/784)	22
All Insertions	MAF≥8	86.9% (619/712)	20
All Insertions	MAF≥10	86.9% (619/712)	20
All Insertions	MAF≥15	95.9% (614/640)	18
All Deletions	MAF≥0	80.5% (3295/4092)	115
All Deletions	MAF≥5	80.5% (3295/4092)	115
All Deletions	MAF≥8	82.6% (3232/3915)	110

Mutation Type	MAF Threshold	Positive Call Rate Among All Observed Mutations	Total Unique Variants
All Deletions	MAF≥10	82.4% (3167/3843)	108
All Deletions	MAF≥15	92.6% (3000/3240)	91

Agreement was also assessed by evaluating the APA and ANA, which assess the degree of agreement for variants based on the average result (**Table 1.20**) APA and ANA across all 3 sites were > 92% for all variant types tested.

Reproducibility was also assessed for independent variables (site, operator, day and within-run). No differences across sources of imprecision were observed.

Table 1.20 Interlaboratory Reproducibility Performance

Alteration Type	Metric	Overall (CI)	Alteration Type	Metric	Overall (CI)
MSI	APA	99.1% (98.7%, 99.4%)	ERBB2 amplifications	APA	100% (99.3%, 100%)
	ANA	99.3% (99.0%, 99.5%)		ANA	100% (100%, 100%)
SNVs	APA	97.8% (97.7%, 97.9%)	ALK translocations	APA	98.6% (97.7%, 99.1%)
	ANA	99.9% (99.9%, 100%)		ANA	99.8% (99.6%, 99.9%)
Insertions	APA	95.6% (95.2%, 96.0%)	NTRK3 translocations	APA	92.7% (90.4%, 94.5%)
	ANA	99.9% (99.9%, 100%)		ANA	99.4% (99.2%, 99.5%)
Deletions	APA	94.4% (94.2%, 94.6%)	RET translocations	APA	98.7% (97.8%, 99.2%)
	ANA	99.9% (99.9%, 100%)		ANA	99.8% (99.6%, 99.9%)
TMB	%CV	3.5%			

The modal positive and negative call rates for sequence mutations (SNVs and indels) in each specimen are summarized in **Table 1.21.**

Table 1.21 Modal Call Rates for Interlaboratory Reproducibility

Specimen	Total Unique Mutations Detected Across All Replicates	Modal Positive Call Rate[1] (n/N) (two-sided 95% CI)	Modal Negative Call Rate[2] (n/N) (two-sided 95% CI)
1	10	99.6% (251/252) (97.8%, 99.9%)	97.2% (105/108) (92.2%, 99.1%)
2[3]	0	-	-

3	9	100% (315/315) (98.8%, 100%)	-
4	7	100% (216/216) (98.3%, 100%)	97.2% (35/36) (85.8%, 99.5%)
5	43	99.5% (1462/1470) (98.9%, 99.7%)	91.4% (32/35) (77.6%, 97.0%)
6	20	98.9% (639/646) (97.8%, 99.5%)	88.2% (30/34) (73.4%, 95.3%)
7	26	97.8% (678/693) (96.5%, 98.7%)	95.2% (157/165) (90.7%, 97.5%)
8	81	96.4% (1991/2065) (95.5%, 97.1%)	88.7% (683/770) (86.3%, 90.8%)
9	88	97.8% (2710/2772) (97.1%, 98.3%)	80.3% (318/396) (76.1%, 83.9%)
10	30	99.0% (998/1008) (98.2%, 99.5%)	97.2%)70/72) (90.4%, 99.2%)
11	94	96.1% (2907/3024) (95.4%, 96.8%)	83.1% (299/360) (78.8%, 86.6%)
12	33	99.4% (1109/1116) (98.7%, 99.7%)	97.2% (70/72) (90.4%, 99.2%)
13	9	100% (216/216) (98.3%, 100%)	93.5% (101/108) (87.2%, 96.8%)
14	24	96.8% (732/756) (95.3%, 97.9%)	88.0% (95/108) (80.5%, 92.8%)

[1]Positive call rate was calculated based on variants with majority call detected as positive.
[2] Negative call rate was calculated based on variants detected at least once, but with majority or equal call as negative. For all other locations, the negative call rates are 100%.
[3] Specimen 2 was selected for presence of ALK translocation and had no detected SNVs or indels.

Precision of MSI was evaluated across 8 MSS and 6 MSI-H samples with a range of MSI scores evaluated by PGDx elio tissue complete with positive call rates provided in **Table 1.22**.

Table 1.22 MSI Performance in the Interlaboratory Reproducibility Study

Case No.	Modal Status	Total Replicates	Mean MSI Score Value	MSI Score Range	SD	%CV	Positive Call Rate (95% CI)
1	MSS	36	10.5	(4.4, 19.1)	3.7	35.1	100% (90.4%, 100%)
2	MSS	35	13.6	(5.8, 20.4)	3.6	26.8	100% (90.1%, 100%)

Case No.	Modal Status	Total Replicates	Mean MSI Score Value	MSI Score Range	SD	%CV	Positive Call Rate (95% CI)
3	MSS	35	13.8	(7.2, 20.2)	3.7	26.8	100% (90.1%, 100%)
4	MSS	36	10.5	(3.9, 19.8)	3.2	30.4	100% (90.4%, 100%)
5	MSI-H	35	209.7	(203.7, 216.5)	3.6	1.7	100% (90.1%, 100%)
6	MSS	34	9.6	(4.7, 16.0)	3.0	31.3	100% (89.9%, 100%)
7	MSS	33	-21.9	(-29.9, -13.2)	5.0	-22.8	100% (89.6%, 100%)
8	MSI-H	35	223.5	(213.1, 236.3)	6.0	2.7	100% (90.1%, 100%)
9	MSI-H	36	271.6	(261.6, 287.2)	5.9	2.2	100% (90.4%, 100%)
10	MSI-H	36	77.5	(62.6, 102.5)	6.6	8.5	100% (90.4%, 100%)
11	MSI-H	36	219.0	(212.6, 224.0)	2.9	1.3	100% (90.4%, 100%)
12	MSS	36	-56.1	(-61.5, -41.5)	4.1	-7.3	100% (90.4%, 100%)
13	MSS	36	16.4	(10.4, 25.1)	3.9	23.7	100% (90.4%, 100%)
14	MSI-H	36	49.3	(36.7, 61.7)	6.3	12.8	94.4% (81.9%, 98.5%)

Precision of TMB was evaluated across 11 samples (with TMB scores above TMB LoB of 7.2 Muts/Mb) with a range of TMB across site, operator, and day provided in **Figure 1.7**.

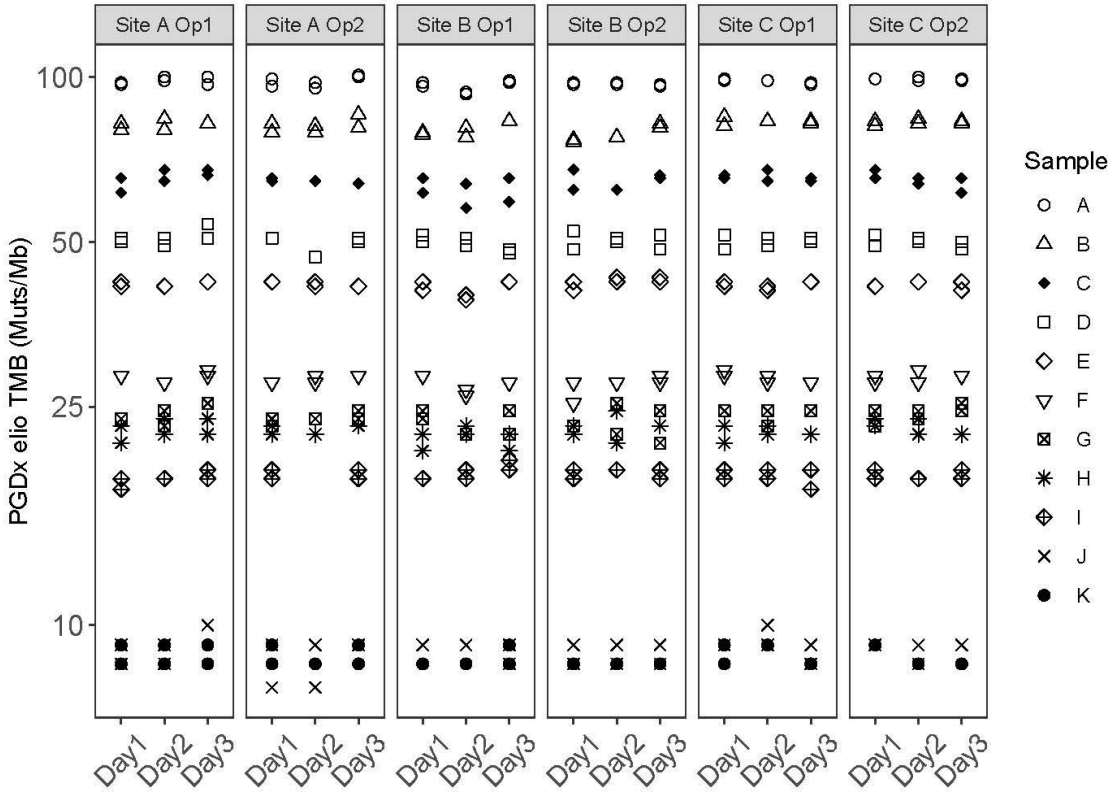

Figure 1.5 TMB Performance in the Interlaboratory Reproducibility Study by Site, Operator, and Day

Lot to Lot Precision

Performance of PGDx elio tissue complete was assessed across 3 unique kit lots by determining concordance of variant calls in FFPE tissue samples. The 3 unique kit lots were utilized to process 5 test cases in triplicate for a total of 45 observations. All batches were sequenced on the same instrument. The overall pass rate of the study was 100% (45/45). **Table 1.23** lists the APA and ANA used to assess lot to lot performance. APA for all variants is > 86%, and %CV for TMB analyses is < 10%.

Table 1.23 Lot to Lot Precision

Variant Type	Performance	Between Lot 1 & 2	Between Lot 1 & 3	Between Lot 2 & 3
Variants with Evidence of Clinical Significance	APA	98.7% (93%, 99.8%)	96.1% (89.2%, 98.7%)	97.4% (91.1%, 99.3%)
	ANA	99.9% (99.6%, 100%)	99.8% (99.4%, 99.9%)	99.9% (99.5%, 100%)
MSI	APA	100% (75.8%, 100%)	100% (75.8%, 100%)	100% (75.8%, 100%)
	ANA	100% (82.4%, 100%)	100% (82.4%, 100%)	100% (82.4%, 100%)

SNVs	APA	92.1% (90.8%, 93.2%)	91.9% (90.6%, 93.0%)	91.9% (90.7%, 93.0%)
	ANA	99.9% (99.9%, 100%)	99.9% (99.9%, 100%)	99.9% (99.9%, 100%)
Insertions	APA	88.9% (80.2%, 94.0%)	88.9% (80.2%, 94.0%)	87.2% (78%, 92.9%)
	ANA	99.9% (99.9%, 100%)	99.9% (99.9%, 100%)	99.9% (99.9%, 100%)
Deletions	APA	86.2% (82.6%, 89.1%)	89.8% (86.7%, 92.2%)	87.3% (83.9%, 90.0%)
	ANA	99.9% (99.9%, 100%)	99.9% (99.9%, 100%)	99.9% (99.9%, 100%)
ERBB2 amplification	APA	100% (61.0%, 100%)	100% (61.0%, 100%)	100% (61.0%, 100%)
	ANA	100% (86.2%, 100%)	100% (86.2%, 100%)	100% (86.2%, 100%)
ALK translocation	APA	100% (61.0%, 100%)	100% (61.0%, 100%)	100% (61.0%, 100%)
	ANA	100% (96.7%, 100%)	100% (96.7%, 100%)	100% (96.7%, 100%)
TMB	%CV	9.5%	7.9%	7.1%

H. Conclusions

The submitted information in this premarket notification the nonclinical and clinical tests that demonstrate that the device is as safe, as effective, and performs substantially equivalent to the legally marketed predicated device and supports a substantial equivalence decision